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D:

Met Gly Val Pro Ile Gly Asp Leu Val Pro Arg Lys Glu Ile Asp Leu
 1 5 10 15
 Glu Asn Leu Tyr Gly Lys Ile Ala Ile Asp Ala Leu Asn Ala Ile
 20 25 30
 Tyr Gln Phe Leu Ser Thr Ile Arg Gln Arg Asp Gly Thr Pro Leu Met
 35 40 45

APPLICANT : AGENCY OF IND SCIENCE &
 TECHNOL;

INVENTOR : KAWASAKI SATOKO;

INT.CL. : C12N 15/09 C07K 14/195 C12N 1/21
 C12N 9/16 // (C12N 1/21 , C12R
 1:19), (C12N 9/16 , C12R 1:19 .)

Cys Asp Glu His Asn Phe Ser Glu Glu Arg Val Lys Asn Gly Ile Glu
 305 310 315 320
 Arg Leu Lys Lys Ala Ile Lys Ala Gly Arg Gln Ser Thr Leu Glu Ser
 325 330 335

TITLE : HEAT-RESISTANT FLAP
 ENDONUCLEASE ORIGINATING FROM
 HYPERTHERMOPHILIC BACTERIUM
 BELONGING TO GENUS
 PYROCOCCUS

Ile Phe Val Lys Lys Iys Pro
 340

ABSTRACT : PROBLEM TO BE SOLVED: To provide the subject new enzyme originating from hyperthermophilic bacterium, consisting of a thermophilic Flap endonuclease having a specific amino acid sequence and high optimum temperature and effective for gene recombination, gene shuffling, etc., based on low homology.

SOLUTION: This protein is a new thermophilic Flap endonuclease composed of the amino acid sequence of the formula and having an optimum temperature of $\geq 75^{\circ}\text{C}$ or a new protein composed of the amino acid sequence of the formula provided that one or several amino acids are depleted, substituted or added and having Flap endonuclease activity. The protein is useful for the gene recombination, gene shuffling, etc., based on low homology. The thermophilic Flap endonuclease can be produced by carrying out PCR reaction with a primer using the chromosome DNA of Pyrococcus horikoshi JCM9974 as a template, integrating the obtained gene into a protein expression plasmid and culturing its transformed E.coli in a medium.

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